# **Original Research Article**

Effects of heating on phytonutrients in cooked aqueous extract of *Vigna unigulculata* (black eyed bean)

#### **ABSTRACT**

**Objective:** To investigate the effects of heating on phytonutrients of cooked *Vigna unigulculata*.

**Methods:** Heating effects on *Vigna unigulculata* by qualitative and quantitative phytochemical analysis, and proximate (nutrient contents) analysis. Five phytochemicals were quantified and nutrient contents determined.

**Results:** Results revealed that phytochemicals in raw sample was higher than cooked sample while proximate analysis of cooked sample showed that crude ash; protein; carbohydrate and crude fiber contents were higher than raw *Vigna unigulculata*.

**Conclusions:** Cooking by heating influenced a reduction of phytochemicals in *Vigna unigulculata*. The increased concentration of phytochemicals in raw *Vigna unigulculata* may obviously be due to the absence of heat action and the high nutritive value of cooked *Vigna unigulculata* could serve as a better source of antioxidants thereby improving healthy life when eaten cooked.

KEYWORDS: Phytonutrients, Black eyed bean, Food content, Health benefits, Extracts

#### Introduction

Vigna unigulculata like other legumes is an essential legume in human nutrition, particularly considered as rich protein and other nutrient source, such as carbohydrates, dietary fiber, minerals and vitamins for the poor of low-income earners, in low-income countries<sup>1,2</sup>. Vigna unigulculata is rich in nutrients. It is composed of minute fat, cholesterol and trans-fat; appreciable amounts of minerals such as iron, potassium and magnesium; vitamins such as folate<sup>3</sup>. Vigna unigulculata like other cowpeas as well has considerable amounts of tannins, phenols and flavonoids, reported to be responsible for its inflammatory modulatory actions<sup>4</sup>. Black-eyed bean is loaded with phytochemicals, which play vital role in fortification of health, prevention of disease and serve as active components in production of drug. Phytochemicals, in their function as antioxidants, excite immune system in humans; stimulate mobilization of protective enzymes in the liver and chunk free radical damage to the gene<sup>5</sup>. Some foods are better eaten unprocessed while others are healthier when cooked. However, for healthy eating, both unprocessed and cooked foods should be eaten to achieve total benefits<sup>6</sup>. Food cooking is reported to destroy food bound enzymes. Enzymes are very sensitive to heat and can be deactivated at temperature above 50°C6. Therefore, for digestion to be complete, the body may need to furnish the process with the required enzymes which may result in enzyme deficiency<sup>6,7</sup>. The various types of food processing by heating such as boiling, steaming, stir-frying and roasting are reported to affect the bioavailability of water soluble vitamins such as vitamin C and B but the fat soluble vitamins are unaffected<sup>8,9</sup>, affect the bioavailability of iron and agonist factors to adequate absorption of mineral<sup>10</sup>. Tannins are not destroyed by cooking in cowpeas but are however slightly lost in the bean soup or broth and a little amount are broken down at cooking 11,12. Thus, raw food may contain more nutrients such as vitamins C and B9. Cooking of food enhances chewing and subsequent digestion of food for easy absorption of nutrients by the body. Weakness of reproductive function and decreased energy are commonly associated with people whose choice is raw-foodist life-style<sup>13</sup>. Cooking legumes such as Vigna unigulculata

helps to diminish the amount of phytate and other anti-nutrients in them. Phytate like other anti-nutrient is capable of hindering plants' nutrients from been absorbed in the body<sup>14</sup>. Half cooked or raw legumes contain precarious toxins known as lectins which can be removed by proper processing of soaking and cooking<sup>6</sup>. Cooking of foods like vegetables has been reported to improve the accessibility of antioxidants phytochemicals such as lycopene, beta-carotene, polyphenols and lutein<sup>15</sup>. Antioxidant functions of lycopene from cooked food is linked to reduced heart disease and reduced risk of prostate cancer, lowers chances of chronic diseases and prevent the body from free radical attack<sup>15</sup>. Cooking of food has been shown to efficiently destroy harmful microorganisms and bacteria that may result in food-borne disease due to improper handling<sup>16</sup>. Thus, for the claim that nutrients in food are lost in cooking, the objective of this present study was to investigate the phytochemicals and food contents in raw and cooked samples of *Vigna unigulculata*, to evaluate the effect of heating on *Vigna unigulculata*.

#### Materials and methods

#### **Materials**

*Vigna unigulculata* (black eyed bean) seeds were purchased from Ogbete main market in Enugu south local government area, Enugu state, Nigeria. The sample was identified and authenticated and a voucher number of UNH no 443 (UNH stands for University of Nigeria Herbarium), was given by Mr. Onyeukwu Chijioke John a plant Taxonomist, Department of Plant Science and Biotechnology, University of Nigeria, Nsukka, Enugu state.

# Preparation of aqueous extract of raw black-eyed bean (rbeb) and cooked black-eyed bean (cbeb)

The black-eyed bean seeds were prepared by winnowing, hand picking of stones, removal of dirt and washed lightly to remove dust and then air dried.

## Preparation of the raw sample

Five hundred gram (500 g) of the dried bean seeds was weighed homogenized into powder and was stored in a clean grease free airtight container with proper labeling for proximate and phytochemicals analysis.

## Preparation of cooked sample

The cooked black eyed bean sample was prepared appropriately by hand picking to remove all foreign particles followed by washing and cooked with enough water until soft and without broth to prevent the loss of some phytochemicals in the bean broth. This was dried under mild sunlight for two weeks under strict supervision. Five hundred gram (500 g) of the dried bean seeds was weighed and homogenized into powder and then stored in a clean grease free airtight container with proper labeling for proximate and phytochemical analysis.

## Preparation of dry extract from samples

From the powdered samples, 200 g was weighed and soaked in 700 ml of distilled water, carefully sealed and allowed to stand for 48 hours (for thorough extraction), before filtering with whatman filter paper. The filtrate was concentrated in water bath at temperature of 70°C.

# Qualitative phytochemical screening of raw black eyed bean (rbeb) and cooked black eyed bean (cbeb) samples

With some modifications on the methods of Harbone<sup>17</sup>; Trease and Evans<sup>18</sup>, nine phytochemicals were identified which include; alkaloids, flavonoids, saponins, glycosides, phenols, steroids, tannins, reducing sugars and anthraquinones in cooked and raw black eyed beans samples.

# Quantitative phytochemical screening of raw black eyed bean (rbeb) and cooked black eyed bean (cbeb) samples

## **Determination of alkaloids**

Alkaloids in the bean samples were determined by the method of Harbone<sup>17</sup>. Five gram (5g) of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and it was covered and allowed to stand for 4 hours at room temperature, and then filtered. The filtrate was concentrated on a water bath to one quarter of its original volume by evaporation and treated with drop wise addition of concentrated aqueous ammonium solution until the alkaloid was precipitated. Alkaloid precipitated was received in a weighed filter paper (W1). It was washed with 1% NH<sub>3</sub> solution and dried in the oven at 80°C. The filter paper and residue was cooled in a desiccator and recorded as (W2). The alkaloid content was calculated and expressed as a percentage of the weight of the sample.

Percentage alkaloid content was calculated using the formula:

$$% Alkaloid = W1 + W2 - W1$$

## **Determination of flavonoid**

Flavonoid was determined by the method of Boham and kocipai-Abyazam<sup>19</sup>. Ten gram (10g) of sample was weighed into a 250 ml conical flask. 100 ml of 80% aqueous methanol added, the mixture was shaken for 3 hours by the aid of electrical shaker. The mixture was filtered into a previously weighed beaker, and evaporated to dryness over a water bath and weighed to a constant.

Percentage flavonoid content was calculated using the formula:

% Flavonoid = 
$$\frac{W2-W1}{W2} \times \frac{100}{1}$$

W1 = weight of empty beaker and W2 = weight of residue (weight of empty beaker + sample after drying).

# **Determination of saponin**

Saponin in raw and cooked samples was determined using 20 method. About 10g of homogenized sample was put into a conical flask and 100ml of 20% aqueous ethanol was added. The mixture was thoroughly mixed for 20 to 30 minutes and it was immediately transferred into a 250ml conical flask. Then, the mixture was covered properly and heated over a hot water bath at 90°C for 4 hours with continuous stirring. The mixture was filtered with whatman filter paper and the supernatant separated. The solid residue was mixed with 100 ml of another 20% ethanol and heated in a similar way for 4 hours. The solution was then filtered and mixed with the previously filtered solution. The combined filtered solution was placed on a hot water bath at 90°C and heated to 20% of the original volume. The concentrated solution was then transferred into a 250 ml separating funnel and 10 ml of diethyl ether was added to it and vigorously mixed. The diethyl layer was discarded carefully after settling down the solution. The purification process was repeated again. Addition of 60 ml n-butanol results in the formation of two layers, the bottom layer was discarded and the upper layer recovered. The combined n-butanol extract was washed twice with 10mls of 5% NaCl solution. The remaining solution (i.e upper layer) was heated in a water bath at 50°C until the solvent evaporated and the solution turned into semidried form. Percentage saponin content was calculated using the formula:

Saponin% = 
$$W_2 - W_1 \times 100$$

 $W_1$  = weight of empty beaker and  $W_2$  = weight of beaker + sample after drying

## **Determination of glycosides**

Glycosides contents in raw and cooked *Vigna unigulculata* were determined by the method of Amadi et al <sup>21</sup>. Five gram (5 g) of the sample was soaked in 100 ml distilled water in a 250 ml conical flask and agitated for 3 hours. The sample was filtered and the total extract was measured and noted. Into a test tube was 2ml of the extract measured and 2ml of 10% DNS reagent was added. Then, the test tube was boiled for 20 minutes in a beaker of boiling water. The test tube was cooled in cold water. The absorbance was read at 540nm using UV-Vis Spectrophotometer, DHG-9101.

Percentage glycoside content was calculated using the formula:

$$\% \text{ Glycoside} = \frac{Absorbance \times total \ volume \ of \ extract \times 100}{1000 \times weight \ of \ sample \ used}$$

## **Determination of tannin**

Tannin in *Vigna unigulculata* samples was determined using the method of Amadi et al <sup>21</sup> with some modifications. Homogenized *Vigna unigulculata* (0.5 g) was weighed into a conical flask and 50ml of distilled water added. After swerving for 1 hour and filtered, 5 ml of the filtrate was pipette into a 50 ml volumetric flask followed by addition of 5 ml of 0.1 % tannic acid. The blank was prepared using 5 ml distilled water in a 50 ml volumetric flask. The three flasks were incubated for one and half hour at 20°C using a water bath and the flasks were made up to 50 ml mark with distilled water. The concentration was determined at 760 nm using UV-Vis Spectrophotometer, DHG-9101.

The concentration of tannin was calculated using the formula:

Tannin (mg/l) = 
$$\frac{X-Y}{Z-Y}$$

X= concentration of extract; Y= concentration of standard (tannic acid); Z= concentration of blank.

## **Proximate analysis**

The proximate analysis of *Vigna unigulculata* samples was done using standard prescription described by<sup>22</sup>.

# **Determination of moisture content**

The moisture content of the raw and cooked samples was determined by the standard method of  $^{22}$ , with the use of hot air oven. Weighing 2-10 g of the homogenized sample into a clean dried petri-dish pre-dried at 98°C for 60 minutes, the sample was dried by heating for a period of 2 to 3 hours to overnight in a hot air oven at  $100^{\circ}$ C. The sample was weighed periodically until it reaches a constant weight. The percent moisture content was calculated from the difference between the initial sample weight (W<sub>I</sub>) and the final sample weight after drying (W<sub>D</sub>). Percentage moisture content was calculated by the formulae;

% Moisture = 
$$\frac{WI - WD}{WI} \times 100$$

W<sub>I</sub> - Initial sample weight; W<sub>D</sub> - Final sample weight

## **Determination of crude ash content**

The method of  $^{22}$  was used for the determination of ash content in raw and cooked samples of *Vigna unigulculata*. A platinum crucible was heated to 600°C in a muffle furnace for 1 hour, and was cooled in a desiccator and weighed as W<sub>1</sub>. Into another crucible, 2 g of the dried sample was

weighed as W<sub>2</sub> and heated at low flame by keeping on a clay triangle until organic matter turns char. The charred material was kept inside the previously set muffle furnace and heated for 6 to 8 hours to greyish white ash and the crucible cooled in a desiccator and weighed as W<sub>3</sub>. The crucible was heated again for further 30 minutes to confirm completion of ashing, cooled and weighed.

Percentage of ash content was calculated using the formula:

% ash content = 
$$\frac{(W3-W1) \times 100}{(W2-W1)}$$

W<sub>1</sub> – Weight of crucible; W<sub>2</sub> - Weight of dry matter with crucible taken for ashing; W<sub>3</sub> – Weight of crucible with ash.

## **Determination of total protein**

The total protein of Vigna unigulculata raw and cooked samples was determined by the standard method described by<sup>22</sup>, with the use of biuret. Series of dilution solution of 0.2 ml, 0.4 ml, 0.6 ml, 0.8 ml and 1 ml of the working standard was pipette into different test tubes. The sample extract (0.5 ml) and 1 ml was pipette into two other test tubes, and made up to 2 ml with distilled water along with the blank tubes. Biuret reagent (3 ml) was added in all tubes, mixed properly and incubated at 37°C for 15 minutes. The colour complex was measured spectrophotometrically at 520 nm.

Concentration of the protein (mg %) = 
$$\frac{OD(test)}{OD(std)} \times \frac{Conc(std)}{Aliquot(test)} \times 100$$

#### **Determination of crude fat**

Crude fat of Vigna unigulculata raw and cooked samples was determined by the standard methods of <sup>22</sup>; Pearson<sup>23</sup>; James<sup>24</sup> using soxhlet apparatus. Weighing 5-10 g of dry sample as W<sub>1</sub> into a thimble and a cotton plug on top of it, the thimble was placed in a soxhlet apparatus and 0.5 ml of ether added to a pre-weighed flat-bottom flask as W<sub>2</sub>, and distilled for 16 hours. The apparatus was cooled and the solvent was filtered into a pre-weighed conical flask. The flask of the apparatus was rinsed with small quantities of ether. The ether was removed by evaporation and the flask was dried with the fat at 80-100°C, cooled in a desiccator and weighed (W<sub>3</sub>).

The percentage of fat content was calculated using the formula:

Fat content 
$$(g/100\%) = \frac{(W3-W2)\times 100}{W1}$$

Where,  $W_1$  – Weight of dry matter taken for extraction;  $W_2$  – Weight of flask bottom flask;  $W_3$  – Weight of flask with flat.

# **Determination of total carbohydrate**

Carbohydrate contents of Vigna unigulculata raw and cooked samples were determined by the method of<sup>22</sup> and nitrogen free extractive (NFE) method described by Pearson<sup>23</sup>. Serial dilution solutions of 0.2 ml, 0.4 ml, 0.6 ml, 0.8 ml and 1.0 ml of the working standard were prepared and pipette into different test tubes. Sample solutions of 0.1 ml and 0.2 ml were pipette into two separate test tubes and each of the test tubes was made up to 1ml with distilled water. Into each tube was 1ml phenol solution added followed by 5 ml of 96% Sulphuric acid and swerved very well. The contents was mixed and placed in a water bath at 25 to 30°C for 20 minutes after 10 minutes and the colour was read at 490 nm. The amount of carbohydrate present was calculated using a standard graph.

The percentage of total carbohydrate present was calculated using the formula:

Absorbance corresponding to 0.1ml of the test = X mg of glucose

100 ml of the sample solution contains =  $\frac{X}{0.1} \times 100$  mg of glucose = % of total carbohydrate present.

#### **Determination of crude fiber**

The crude fiber of raw and cooked samples of *Vigna unigulculata* was determined by the method of  $^{22}$ . Two gram (2 g) of the dried sample was boiled with 200 ml Sulphuric acid for 30 minutes with bumping chips and filtered through muslin and washed with boiling water until washings were no longer acidic. The residue was boiled with 200 ml of Sodium hydroxide solution for 30 minutes and filtered through a muslin cloth and washed with 25 ml of boiling 1.25%  $H_2SO_4$ , three 50 ml portions of water and 25 ml ethanol. The residue was removed and transferred to a pre weighed ashing dish  $(W_1)$  and was dried for 2 hours at  $130 \pm 2^{\circ}C$ . The dish was cooled in a desiccator and weighed as  $W_2$ . The dish was heated again for 30 minutes at  $600 \pm 15^{\circ}C$ , cooled in a desiccator and reweighed as  $W_3$ .

The percentage content of crude fiber in sample was calculated using the formula:

% Crude fiber in sample = 
$$\frac{Loss \ in \ weight \ on \ ignition \ (W2-W1)-(W3-W2)}{Weight \ of \ the \ sample} \times 100$$

#### Statistical analysis

All the analyses were performed in triplicate and average values calculated were expressed according to required units. Analysis of variance (ANOVA) with the IBM statistical package for social sciences (SPSS) for Windows version 23, was used to analyze collected data. The Bonferroni post hoc test was used to identify the means that differ significantly at p<0.05. Results are presented as mean  $\pm$  standard deviation.

## **Results and Discussion**

The phytochemical qualitative screening (Table1) of aqueous extracts of both samples showed that alkaloids were very deeply present (+++) in RBEB and deeply present (+++) in CBEB; Flavonoids were not detected (ND), Frothing Saponins were very deeply present (+++) in RBEB and not detected in CBEB and emulsion form of saponins were deeply present (+++) in CBEB and not detected in RBEB; Cyanogenic glycosides were very deeply present (+++) in RBEB but deeply present (+++) in CBEB; Cardiac glycosides were not detected in both samples; Phenols were very deeply present (+++) in both samples; Steroids were not detected in both samples; Tannins were deeply present (+++) in RBEB but not detected in CBEB; Reducing sugars and anthraquinones were not detected (ND) in both samples and Terteoids were deeply present (++) in RBEB and CBEB.

Table 1: Results of Qualitative Phytochemical Analysis of RBEB and CBEB Samples

S/N	PARAMETER	RBEB	CBEB
1.	Alkaloids	+++	++
2.	Flavonoids	ND	ND
3.	Glycoside		
	(a) Cyanogenic	+++	++
	(b) Cardiac	ND	ND
4.	Phenols	+++	+++
5.	Steroid	ND	ND
6.	Tannins	++	ND
7.	Reducing Sugar	ND	ND
8.	Anthraquinone	ND	ND

9.	Terteoids	++	++
10.	Saponins		
	i. For Frothing	+++	ND
	ii. For Emulsion	ND	++

Keywords: Very deeply present (+++), deeply present (++), present (+), and not detected (ND), Raw black eyed beans (RBEB) and Cooked black eyed beans (CBEB).

Results of quantitative analysis of CBEB and RBEB in Table 2 revealed the following amount of phytochemical in a decreasing order distribution: Alkaloids in RBEB ( $16.5\pm0.49\%$ ) > CBEB ( $8.85\pm0.06\%$ ); Flavonoids in RBEB ( $10.01\pm0.01\%$ ) > CBEB ( $1.16\pm0.01\%$ ); Saponins in RBEB ( $3.18\pm0.01\%$ ) > CBEB ( $2.13\pm0.01\%$ ); Tannins in CBEB ( $1.05\pm0.017$  mg/l) > FBEB and Glycoside in CBEB ( $1.51\pm0.01\%$ ) > RBEB ( $1.52\pm0.02\%$ ).

Table 2: Results of Quantitative Phytochemical Analysis of RBEB and CBEB Samples

S/N	PARAMETER	RBEB	CBEB
1.	Alkaloids %	16.5±0.49	8.85±0.06
2.	Tannins (mg/l)	ND	1.05±0.017
3.	Saponins %	3.18±0.01	2.13±0.01
4.	Flavonoids %	10.01±0.01	1.16±0.01
5.	Glycosides %	1.51±0.01	1.52±0.02

Results are Mean  $\pm$  Standard deviation for duplicate analysis; the mean difference is significant at P<0.05. Keywords: Raw black eyed beans (RBEB) and cooked black eyed beans (CBEB).

The proximate analysis of the bean samples is shown in Table 3 below. The results showed that moisture content of RBEB (9.47 $\pm$ 0.121%) was higher than CBEB (4.98 $\pm$ 0.222%); ash content of CBEB (14.25 $\pm$ 0.002%) was higher than RBEB (12.06 $\pm$ 0.003%); protein content of CBEB (7.92 $\pm$ 0.342%) was found to be higher than RBEB (9.06 $\pm$ 0.752%); crude fiber of CBEB (8.39 $\pm$ 0.001%) was higher than RBEB (6.29 $\pm$ 0.463%); crude fat of RBEB (13.23 $\pm$ 0.294%) was found to be higher than CBEB (7.92 $\pm$ 0.342%) and carbohydrate content of CBEB (95.47 $\pm$ 0.468%) was found to be higher than RBEB (63.94 $\pm$ 0.588%).

Table 3: Results of proximate analysis of RBEB and CBEB Samples

S/N	PARAMETER	RBEB	CBEB
1.	Moisture content %	9.47±0.121	4.98±0.222
1.	Crude Ash Content %	$12.06 \pm 0.003$	$14.25 \pm 0.002$
2.	Crude fat%	$13.23 \pm 0.294$	$7.92 \pm 0.342$
3.	Protein Content%	$9.06 \pm 0.752$	65.66±0.302
4.	Crude fiber%	$6.29 \pm 0.463$	$8.39 \pm 0.001$
5.	Carbohydrate%	$63.94 \pm 0.588$	$95.47 \pm 0.468$

Results are Mean  $\pm$  Standard deviation for duplicate analysis; the mean difference is significant at P<0.05. Keywords: Raw black eyed beans (RBEB) and cooked black eyed beans (CBEB).

#### **Discussion**

Vigna unigulculata seed is a nutritious food with high contents of rich phytochemicals and proximate properties. However, methods of processing may contribute to the unavailability and availability of these nutrients and phytochemicals<sup>1</sup>. Quantification of some of the phytochemicals showed that alkaloids content was higher followed by flavonoids and then saponins in raw Vigna unguiculata while tannin content was higher followed by cyanogenic glycoside in cooked Vigna unguiculata. This is consistent with the findings of Idoko et al<sup>25</sup>, were alkaloids in cooked Phaseolus vulgaris was lower. Alkaloid content in raw sample was higher than cooked Vigna *unguiculata* in this study. Alkaloid was reported to be high also in *Balanites aegyptiaca* kernel<sup>26</sup>. Alkaloids applications in medicine are reported to be spectacular in their physiological functions due to their non toxicity<sup>27</sup>. The pharmacological properties of alkaloids are reported to include hypoglycaemic, hypotensive, analgesic and anti-tumor properties<sup>28</sup>. Tannin content in cooked sample was more than the raw sample. This is inconsistent with the findings of Jasraj and Kiran<sup>29</sup>, who reported that household cooking methods such as pressure cooking and boiling, significantly destroyed antinutrients in Vigna unguiculata. Tannins, trypsin and phytate have been known to be antinutrients in most legumes. Thus, these phytochemicals in Vigna *unguiculata* are likely not reckon with any nutritional value<sup>29</sup>. Tannins and other antinutrients in legumes, as inhibitors to protein digestion are said to be destroyed by cooking thereby increasing protein digestion and its quality and also promote the functions of protease and amylase<sup>30</sup>. Antinutrients are higher in raw plants' foods and consuming raw foods make these antinutrients to impede metabolic process. Thus, from this study, it becomes imperative to thoroughly soak, cook, fry and boil legumes and some plants' food to eliminate antinutrients<sup>29</sup>.

In this study, saponin content was higher in raw sample than in cooked sample. However, the saponin content in this study of both samples is lower than that reported by<sup>26</sup>. A very high saponin level is reported to result in gastroenteritis linked dysentery and diarrhea<sup>31</sup>. Saponins are greasy and bitter taste phytochemicals with glycoside bonds found abundantly in plants<sup>31</sup>. The hepatoprotective, hypoglycaemic, hypolipidaemic, anti-inflammatory, anti-diabetic and anti-HIV potentials of saponins have been reported<sup>28</sup>.

The amount of flavonoids in raw *Vigna unguiculata* was higher than that in cooked *Vigna unguiculata*. This is contrary to the report of Idoko et al.<sup>25</sup>, were cooked *Phaseolus vulgaris* was higher. The higher value of flavonoids in raw sample over cooked could be attributed to the claim that higher temperature is capable of destroying volatile bionutrients and therefore reduce their quantity<sup>6</sup>. Flavonoids abound in many plants and they contribute immensely to the color and flavor widespread variety of beans<sup>32</sup>. The six subclasses of flavonoids reported to be found in beans include, anthocyanins, flavanones, isoflavonoids, flavanols, flavonols and flavones. Hesperetin glycosides and naringenin are the two most important flavanones among the nine branded flavanones in widespread bean types reported<sup>32</sup>. Huber et al<sup>33</sup> reported that heat action on beans increased the antioxidant activities and the concentrations of phenolic compounds. Flavonoids in beans are known for their antioxidant and pharmacological activities in human health, this include; anti-inflammatory, anti-carcinogenic, anti-mutagenic, antimicrobial, anti-diabetic, anti-allergic and anti-diarrheal activities<sup>28</sup>. However, the flavonoid content of both samples in this study was found to be lower than that reported by Idoko et al.<sup>25</sup> and Huber et al<sup>33</sup> in *Phaseolus vulgaris*.

The percentage proximate composition of cooked black eyed bean has higher ash content than that of raw black eyed bean. This result is contrary to what was reported for boiled *Vigna unguiculata* by Omenna et al<sup>34</sup> and for boiled *Vigna Sesquipedalis*, which was reduced by 21% <sup>35</sup>. Ash content of an organic matter presents a brilliant indicator for its nutritional value and

mineral content measurement and therefore better yield of biogas and biofertilizer<sup>36</sup>. Thus, high ash content of cooked *Vigna unguiculata* sample suggests that cooking makes the valuable minerals and nutrients much available<sup>6</sup>. The crude fat in raw black eyed beans is quite higher than that of cooked black eyed beans. This is consistent with the report of Nzewi and Egbuonu<sup>35</sup> were boiling was found to reduce crude fat in *Vigna Sesquipedalis*. Crude fat in *Balanites aegyptiaca* seed oil was said to be a good source of liquid cleansing agent and biofuel Ubwa et al<sup>36</sup> and it was reported to have several medicinal application<sup>38</sup>.

Crude protein content of raw sample was found to be lower than the cooked sample. The higher content of crude protein in cooked Vigna unguiculata was different to the report of <sup>39</sup>, were protein content was found to be reduced when Phaseolus Vulgaris bean seed was cooked. However, the higher protein content in cooked bean sample could be due to complete destruction and elimination of antinutrients which would have interfered with protein<sup>6</sup> and also support the claim that Vigna unguiculata and other legumes are proteinous and thus, the reason low income earners depend on it for protein source<sup>2</sup>. In this sense, *Phaseolus Vulgaris* (raw and cooked), was reported to possess potential of improved kidney function in albino wistar rats, attributed to its healthy nutrients contents, especially protein<sup>25,40</sup>. Similarly, crude fiber in cooked sample was higher than raw sample of Vigna unguiculata. High crude fiber content in cooked black eyed beans could improve bowl movement and eliminate constipation. This would possibly reduce the often associated allergic reaction to beans consumption<sup>41</sup>. The moisture content of cooked Vigna unguiculata was found to be lower than the raw sample, which was similar to that reported by 35. Low moisture content may reduce microbial activity, enhances and elongate storage and reduce free fatty acids and low acid value 42,43. The content of crude carbohydrate was higher in cooked Vigna unguiculata than the raw sample. This was consistent with the report of Omenna et al<sup>34</sup>, who observed that pressure cooking of Vigna unguiculata bean seed yielded higher crude carbohydrate than the raw sample and boiling Vigna Sesquipedalis for 40 minutes increased carbohydrate content by 8% 35. The level of resistant starch was found to be increased after cooked legume was cooled for 24 hours in the refrigerator, which resulted in recrystalization of the starch molecules<sup>41</sup>. The proximate composition of the cooked black eved beans of this study indicates it is highly nutritious as it contains high protein content, hence could supplement other protein sources such as, peas and groundnuts and could increase protein composition when cooked with rice especially in dry seasons and in arid regions<sup>2</sup>. Therefore, the continuous rise in the cost of animal proteins like meat, egg, fibre and milk could be reduced by processing this cooked beans and used as a protein supply for both humans and animal's nutrition.

#### **Conclusion**

From this investigation, it may be concluded that cooking of *Vigna unigulculata* bean seed improves its protein content, carbohydrate content, ash content and fiber content, and therefore makes its consumption safer with better antioxidant effect. However, phytochemicals in *Vigna unigulculata* that could not withstand heat were found to be reduced in the cooked sample and the high nutritive value of cooked *Vigna unigulculata* could serve as a better source of antioxidants thereby improving healthy life when eaten cooked.

#### **CONFLICT OF INTEREST**

No conflict of interest exists between authors as it relates this work.

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